LOCO: Characterization of Phytoplankton in Thin Optical Layers

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Award Number: N000140410275
http://thalassa.gso.uri.edu/rines/loco/index.html
http://thalassa.gso.uri.edu/rines
http://www.gso.uri.edu/criticalscales

LONG-TERM GOALS

Our long-term goal is to understand the ecology of phytoplankton inhabiting coastal shelves, upwelling areas, fjords and banks. We are especially interested in ways in which species-specific properties, including colony size and shape (diatoms) and motility (dinoflagellates) interact with physical mixing processes to regulate spatio-temporal distribution patterns. We wish to understand these processes in sufficient detail to be able to predict bloom dynamics, size structure, and the impact of species-specific characteristics of the phytoplankton on ocean optics.

OBJECTIVES

Our goals within the LOCO DRI program are (1) to thoroughly characterize the phytoplankton community within thin layers and compare it to that outside of layers, (2) to increase our understanding of the importance of species-specific characteristics of the plankton to both ecology and ocean optics, and (3) to expand our understanding of the role that biological-physical processes play in thin layer dynamics.

APPROACH

Under previous ONR funding (N000149610247, N000140210247), we have demonstrated that interactions between physical processes at multiple time and space scales, and the species-specific properties of diatoms and dinoflagellates (*e.g.* size, shape, behavior etc.) are important factors contributing to phytoplankton distribution, bloom dynamics, particle size structure and optical characteristics in the ocean. In order to continue this work within the LOCO framework, we have (1) adapted our earlier protocols for use in the open waters of Monterey Bay (*i.e.* exposed, coastal locations), and (2) developed methodologies that will allow us to collect new kinds of data, so that we can begin to investigate our 'next generation' of questions. In August/September of 2005, and in July 2006, we employed our refined protocols during the LOCO field experiments in Monterey Bay, California. Our primary effort was carried out in close collaboration with Donaghay, Sullivan, Holliday and Hanson, working from *R/V Shana Rae*. We are fortunate to also have the opportunity to collaborate with the many additional PIs in the LOCO program.

Report Documentation Page

Form Approved
OMB No. 0704-0188

1. REPORT DATE	2. REPORT TYPE	3. DATES COVERED
2006	**N/A**	**-**

4. TITLE AND SUBTITLE	5a. CONTRACT NUMBER
LOCO: Characterization of Phytoplankton in Thin Optical Layers	5b. GRANT NUMBER
	5c. PROGRAM ELEMENT NUMBER
6. AUTHOR(S)	5d. PROJECT NUMBER
	5e. TASK NUMBER
	5f. WORK UNIT NUMBER
7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES) **Graduate School of Oceanography University of Rhode Island South Ferry Road Narragansett, Rhode Island 02882-1197**	8. PERFORMING ORGANIZATION REPORT NUMBER
9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES)	10. SPONSOR/MONITOR'S ACRONYM(S)
	11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION/AVAILABILITY STATEMENT
Approved for public release, distribution unlimited

13. SUPPLEMENTARY NOTES
The original document contains color images.

14. ABSTRACT

15. SUBJECT TERMS

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	b. ABSTRACT	c. THIS PAGE			
unclassified	**unclassified**	**unclassified**	**UU**	**9**	

WORK COMPLETED

Our efforts in FY07 were divided between analysis of samples collected during the 2005 and 2006 LOCO field experiments, and preliminary evaluation of the capabilities of our new CytoSense scanning, in-line, bench-top/*in situ* flow cytometer - http://www.cytobuoy.com/

LOCO Sample Analysis. Our sample set from the LOCO experiments includes over 300 preserved whole water samples collected from inside and outside thin layers and surface slicks, about 30 offshore samples from *R/V New Horizon* (2005), live counts (2005) of the fragile dinoflagellate *Akashiwo sanguinea* (which does not reliably preserve), over 100 filter samples for epifluorescence-based image analysis of picoplankton, over 20 hours of videotaped record of microscopic examination of live phytoplankton, and about 70 CytoSense samples (2006). Analysis of samples has been prioritized to address specific questions, and to interact with other members of the LOCO team. Preliminary analysis of 2005 samples is largely complete; additional analyses will be done as needed for specific papers. Of the 2006 samples, about 75% of the picoplankton filters have been analyzed, 35% of the whole water samples have been counted, and 90% of the CytoSense samples have been processed.

CytoSense/CytoSub Evaluation. In May, Rines and McFarland traveled to CytoBuoy headquarters in Nieuwerbrug, the Netherlands. Our objectives were to obtain training on our instrument, and to collaborate with CEO Mr. George Dubelaar and his staff to identify ways in which to optimize the instrument to best address our research interests. Our trip was very successful! We are currently conducting a series of laboratory experiments designed to allow us to quantitatively evaluate the performance of the instrument so that we can both correctly interpret the CytoSense data collected during the LOCO 2006 field experiment, and begin to design future laboratory and field efforts.

RESULTS

LOCO. Monterey Bay is home to an extraordinarily diverse community of phytoplankton. Although many of the same taxa were present in both 2005 and 2006, the dynamics of each year were quite different, and were correlated to hydrographic patterns. In 2005, the absence of upwelling winds led to a stratified water column throughout the experiment. Dinoflagellates were prevalent in the upper, and diatoms in the lower regions of the water column. The most striking feature of the community was a large population of the photosynthetic dinoflagellate *Akashiwo sanguinea*, which was located near the surface or in surface slicks during the day, and migrated downward at night, forming an intense thin layer at the pycocline/nutricline. In 2006, the hydrography was far more complex. LOCO researchers identified 3 successive hydrographic regimes. Each had its own phytoplankton community pattern. Regime I: The water column was well mixed, with a lot of advection and low stratification. Offshore winds were the highest of the experiment. Patchy surface slicks contained the photosynthetic ciliate *Myrionecta rubra*, and the dinoflagellate *Alexandrium catenella*. The water column was dominated by the diatom genus *Pseudo-nitzschia*. Regime II: Winds were low both onshore and offshore. There was little mixing, and stratification built. Increased sunshine led to thermal warming at the surface. We frequently observed thin layers during this period. A layer around 3m depth was dominated by *Myrionecta rubra*, the dinoflagellates *A. catenella* and *Dinophysis fortii*, and other flagellates. A layer commonly observed around 5m was dominated by the dinoflagellates *Ceratium lineatum* and *C. furca*, and a variety of diatoms. Regime III: Offshore winds built, tidal height increased, and the continued thermal warming maintained stratification. A mixed diatom community was present in the upper waters, and a large population of *Chaetoceros concavicornis* was present at depth. Surface slicks were observed throughout the experiment. Unlike 2005, different patches were dominated by different

species. A remarkable feature of the 2006 phytoplankton community was the prevalence of potentially toxic species known to form Harmful Algal Blooms (Figure 1).

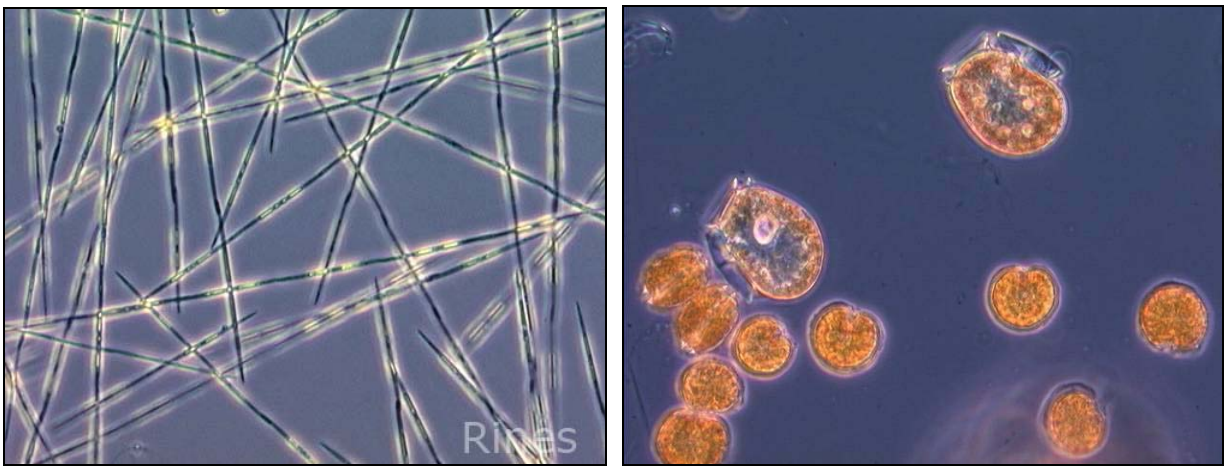

Figure 1. Photomicrographs of some of the Harmful Algal Bloom taxa observed in 2006. Left, Pseudo-nitzschia, which was found throughout the water column during Regime I. Right, Dinophysis and Alexandrium from a thin layer located at ~3m depth during Regime II.

Picoplankton (~ 0.5-10 µm) vertical distribution and community structure also varied in space and time. In last year's report, we depicted a typical pattern for 2005, in which picoplankton varied independently of net plankton, were enhanced near the surface, and were not concentrated in thin layers. Yellow fluorescing *Synechococcus* were dominant. In 2006, we observed additional kinds of patterns. For example, during the *Pseudo-nitzschia* bloom observed during Regime I, picoplankton followed a similar distribution to that of the diatoms (Figure 2), consistent with the hydrographic pattern of a well-mixed water column. *Prochlorococcus* and picoeukaryotes were dominant.

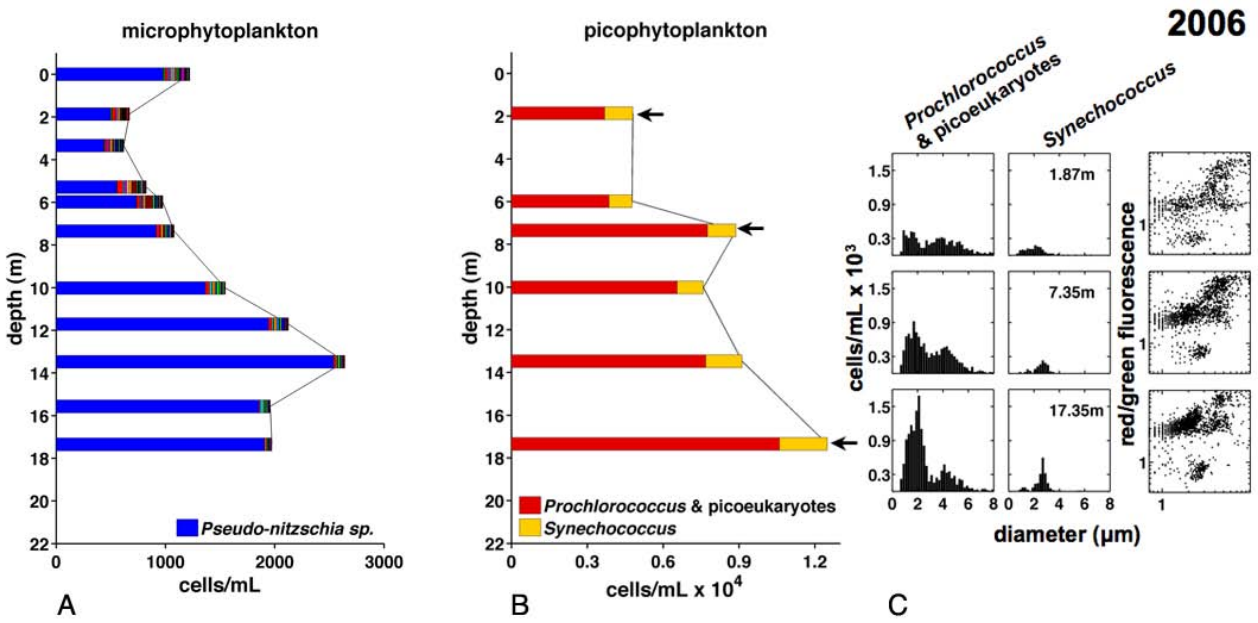

Figure 2. Microphytoplankton and picophytoplankton distribution during Regime I of 2006. A. Microplankton are dominated by Pseudo-nitzschia (blue), and their concentration gradually increases with depth. Other colors represent additional taxa. B. Picoplankton show a similar distribution. C. Size-frequency distribution of picoplankton for the three depths indicated by arrows. Scatter plots separate the population into clusters based on pigments and size classes.

CytoSense Evaluation. Our CytoSense scanning, in-line flow cytometer was specifically designed to study the size, shape, physiological and optical properties of phytoplankton colonies and individual cells within the colonies. It can be used both bench-top, and *in situ*. It streams near-real-time, multi-channel data on the size and optical properties of each particle as it flows past the sensors, creating a detailed scan of the variations in complexity of each parameter over the length of the particle. Our instrument contains a blue (488nm) laser, and sensors to measure forward scatter, side scatter, red, orange, yellow and green fluorescence, and curvature. It is the first of a new generation of high data speed (USB) CytoSense instruments. We have both the hardware and software up and running, and are working with the CytoBuoy staff on its continuing evolution. We have recently grown cultures of three large, chain-forming diatoms of radically different morphology for initial characterization, two of which are depicted in Figure 3. *Cerataulina dentata* forms hair-like colonies up to about 1cm in length composed of hundreds of lightly silicified cylindrical cells. *Stephanopyxis turris* is quite heavily silicified, and its frustules are composed of complex areolae, and interlocking spines. It formed colonies a few mm in length. *Chaetoceros compressus* var. *hirtisetus* exhibits differentiation within a colony, and torsion about the colony axis. Its colonies were typically ~ 500 µm in length.

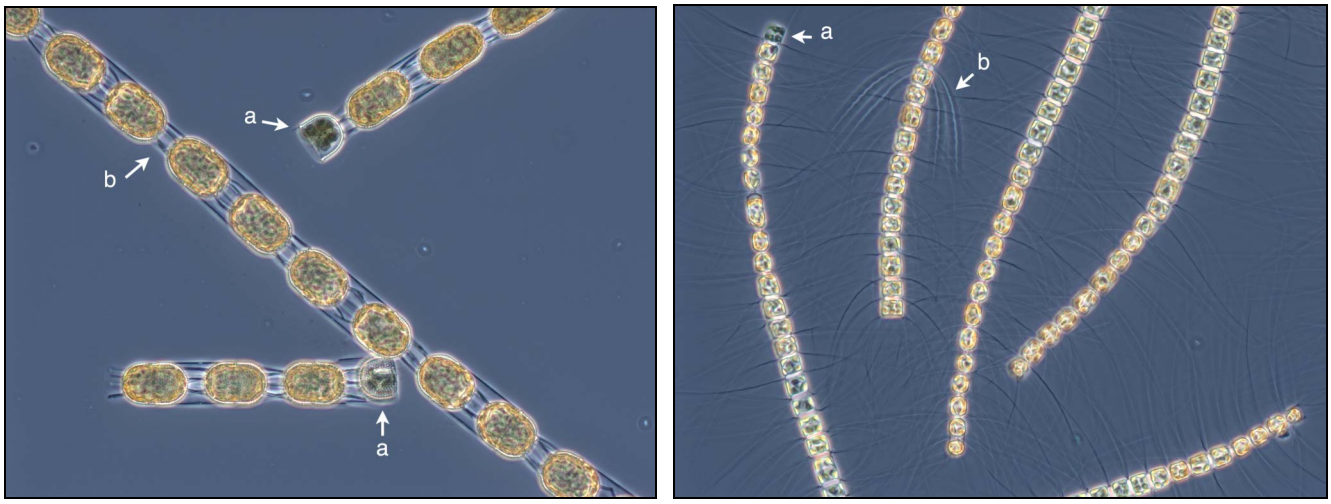

Figure 3. Photomicrographs of two examples of colonies studied with CytoSense. Left, Stephanopyxis turris. a, broken cells with dead contents. b, siliceous linking spines that unite cells into colonies. Right, Chaetoceros compressus var. hirtisetus, with numerous fine siliceous setae. a, broken cell with dead contents, b, heavily silicified intercalary setae.

These three species of diatoms produced quite different pulse profiles when run through CytoSense. In addition to species-specific differences, variations within a population are also apparent, indicating that the instrument can document a wealth of detailed information on morphology, physiological condition (as chlorophyll fluorescence) and optical properties of phytoplankton at many hierarchical levels (*i.e.* between species, between individual colonies of a given species, sub-colony structure, sub-cellular structure). For example, Figure 4 depicts the pulse profile for a gently curved, 1.6 mm colony of *Stephanopyxis*, composed of about 31 cells, with a dead cell at one end of the chain. Figure 5 depicts data from a much shorter colony, and the expanded x-axis reveals that there is considerable within-cell variation in forward scatter. We suspect that this is related to the complex, three dimensional areolar structure of the frustule, which is visible with light microscopy, but best visualized with electron microscopy.

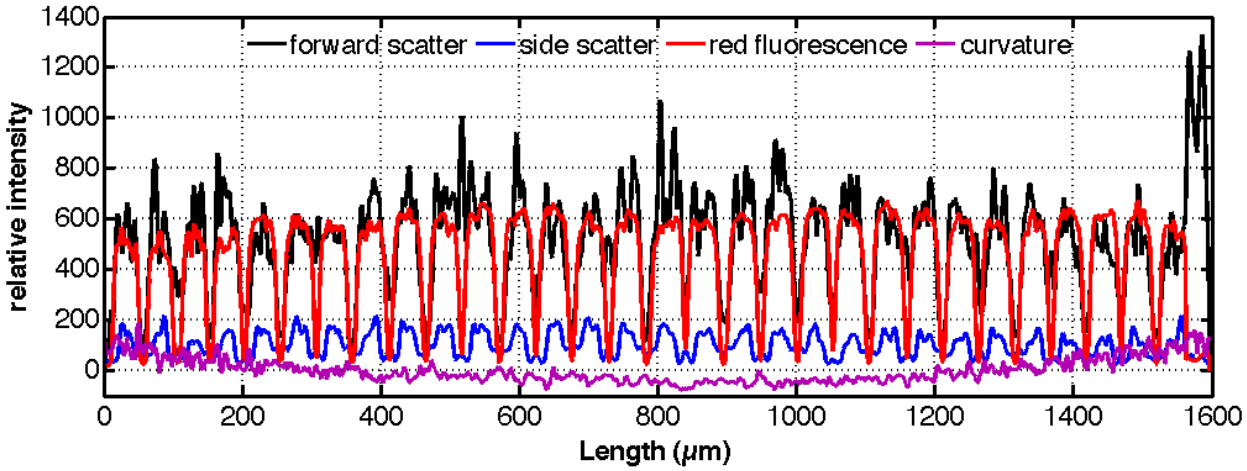

Figure 4. Pulse profile of a 1.6 mm colony of Stephanopyxis turris, showing forward scatter, side scatter, red (chlorophyll) fluorescence and curvature. At the extreme right edge of the plot is the signature of a dead cell, characterized by no chlorophyll, and high scatter of the empty siliceous shell. This suggests a broken chain, similar to that depicted in the photomicrograph in Figure 3. Note that curvature oscillates above and below the 0 x-axis, indicating that the chain is gently arced.

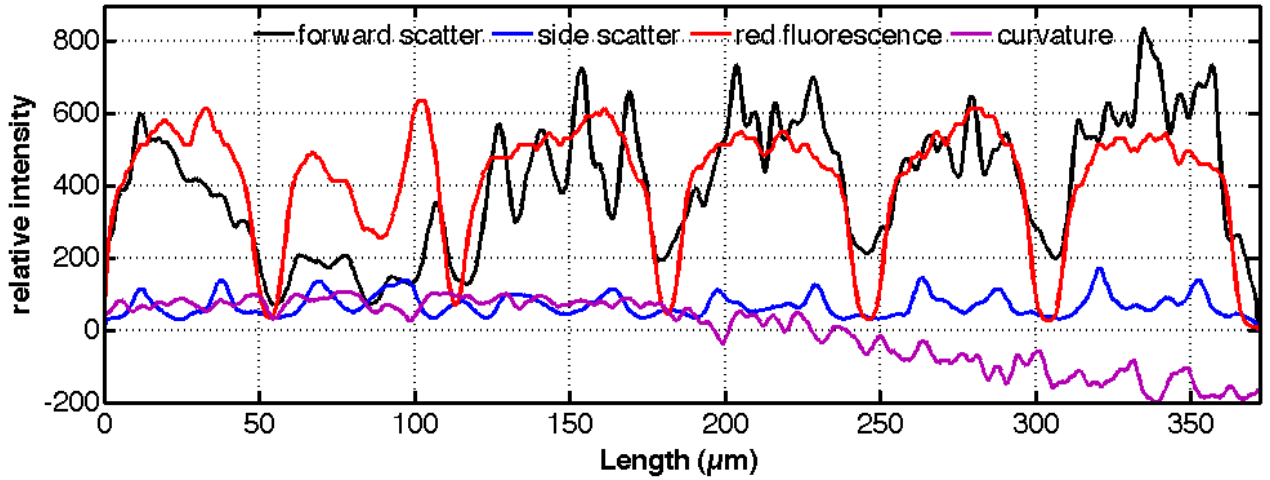

Figure 5. A 350 µm colony of Stephanopyxis turris. Each set of red and black pulses indicates an individual cell. The expanded x-axis allows one to see that there is a large amount of within-cell variation in forward scatter (black line).

In contrast, *Chaetoceros compressus* var. *hirtisetus* shows little within-cell variation in forward scatter (Figure 6), although within-colony variation may depict cellular differentiation within a colony, such as the formation of specialized setae. In order to demonstrate that CytoSense can be used to study the physiological state of a population (as indicated by chlorophyll fluorescence), we continued one experiment until the population became senescent, and microscopic examination revealed the presence of many dead cells within the colonies, as is often seen a the end of a natural phytoplankton bloom. In

a representative pulse profile from this material (Figure 7), it is possible to count the number of dead cells within a chain, and determine their position along the chain axis.

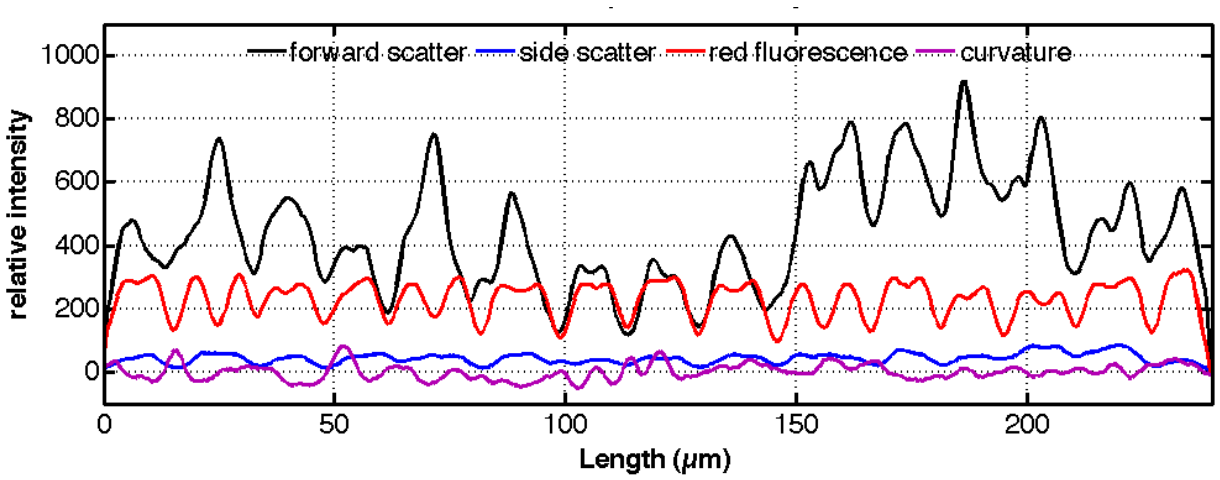

Figure 6. A pulse profile of a healthy chain of Chaetoceros compressus var. hirtisetus. The elevated intensity of forward scatter between 150 and 200 μm length may indicate the presence of a set of heavily silicified intercalary setae (compare to the image in Figure 3).

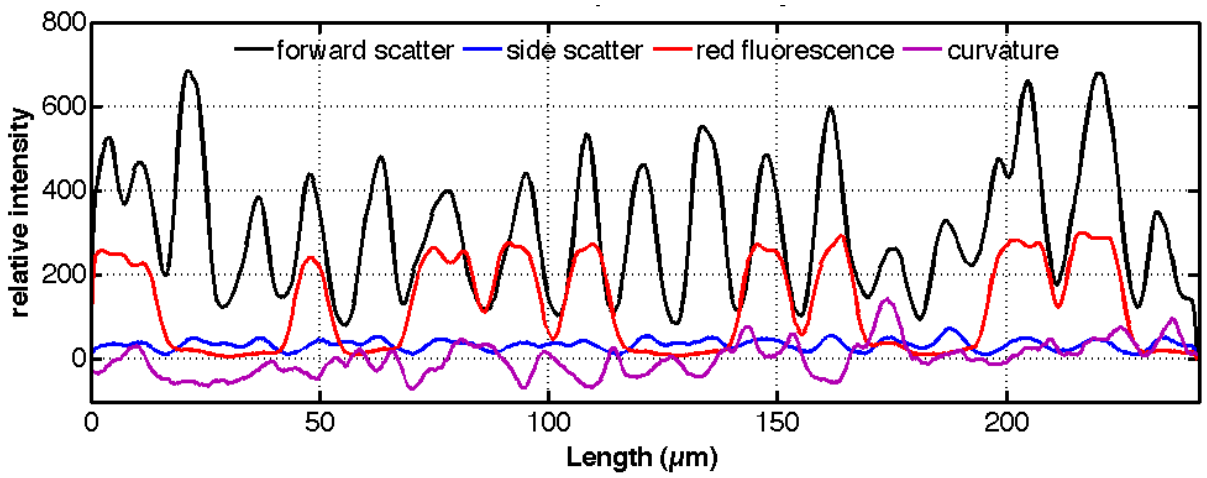

Figure 7. A pulse profile from the same culture of Chaetoceros compressus var. hirtisetus several days later, after it had reached senescence. Note the many dead cells, which contain no chlorophyll.

Diatom colonies grown in our experimental tanks were visible by eye, and achieved lengths on the scale of millimeters. Similarly large colonies have been documented in the field with photomicrography (Rines), and submersible holography (Katz *et al.* 1999, Malkiel *et al.* 1999). We are in the process of compiling comparative length-frequency information on each cultured population based on image analysis of colonies photographed *in situ* with macrophotography, and analysis by

CytoSense. Our preliminary data suggests that some breakage of large particles is occurring, and we are currently investigating whether this is a calibration issue, actual breakage of material within the instrument, or a function of the hydrodynamics of the intake port.

IMPACT/APPLICATIONS

Thin Layers of phytoplankton are an important feature of the coastal ocean. However, they don't exist in isolation – they are a component of the biological and hydrographic dynamics of the entire water column, and must be studied as such. Thin layers may simply contain an enhanced concentration of the phytoplankton community found throughout the water column, but frequently they contain a unique flora, with layers at different depths dominated by different taxa. Patterns exist at multiple scales. In addition to species-specific differences, we have demonstrated that *groups* of organisms (*e.g.* diatoms, dinoflagellates and picoplankton) can exhibit separate patterns of vertical distribution, thus different processes must regulate their dynamics. These relationships are not static: layers of motile organisms may migrate in and out of other structures. Thus, there may be many simultaneously occurring and interacting patterns, operating on multiple spatiotemporal scales.

Our LOCO data provides a wealth of information on the species-specific distribution of phytoplankton inside and outside of thin layers in Monterey Bay. When collaboratively combined with the physical, chemical, optical and acoustical data of our LOCO colleagues, we have a unique opportunity to further our knowledge of both the mechanisms of thin layer formation, maintenance and dissipation, and the biological, ecological and optical impacts of those layers.

Species-specific properties of phytoplankton such as size, shape, pigment composition, biomineralization and toxin production are known to play important ecological and oceanographic roles. However, the classical 'form and function' questions remain largely unanswered (Sournia 1982), and to my mind are amongst the most fascinating in biological oceanography. I am especially interested in the interactions between phytoplankton morphology (at both colony, and subcellular levels), physical mixing processes operating at the scale of the organism, and optics. Our CytoSense flow cytometer gives us a new, innovative tool with which to pursue the significance of particle variability with respect to biological/ecological questions, and also from the perspective of impact of species-specific properties of the phytoplankton on ocean optics. This instrument does not replace a microscope – its tremendous power lies in generating data to link IOPs to the highest quality, detailed microscopic images that we can obtain of the organisms themselves. Our initial impression of the data collected suggests that CytoSense can quantify the optical properties of plankton is such a detailed way that it will both revolutionize our studies of phytoplankton ecology, and provide data critical to linking microscope-based studies of the species-specific properties of phytoplankton to the *in situ* inherent optical properties (IOPs) measured by colleagues Donaghay & Sullivan.

RELATED PROJECTS

We are working closely with Donaghay & Sullivan to link species-specific patterns of plankton distribution to physical and optical data. We are working with D.V. Holliday (BAE Systems) to provide photomicrographs, with size information, of zooplankton samples collected by Holliday & Donaghay via pumping, and net tows from *R/V Shana Rae*. We will collaborate with A.K. Hanson to correlate phytoplankton and nutrient distributions. We will collaborate with J. Ryan (MBARI) to compare the composition of surface samples to overflight data. We expect to find many additional opportunities to collaborate closely with other colleagues funded in the ONR LOCO Program.

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Malkiel, E., O. Alquaddoomi & J. Katz (1999) – Measurements of plankton distribution in the ocean using submersible holography. Meas. Sci. Technol. 10: 1142-1152.

Sournia, A. (1982) – Form and function in marine phytoplankton. Biological Revue 57: 347-394.

PRESENTATIONS

Rines, J., M.N. McFarland, P.L. Donaghay, J.M. Sullivan & J.R. Graff. Importance of species-specific characteristics of the phytoplankton to the dynamics and properties of thin layers during the Monterey Bay LOCO experiment, 2006. ASLO, Santa Fe, February 2007

McFarland, M. & **J. Rines.** Automated image analysis of autotrophic picoplankton. Northeast Algal Society, April 2007.

McFarland, M.N., **J. Rines**, P. Donaghay & J. Sullivan. Fine scale distribution and abundance of large and small phytoplankton in Monterey Bay, CA. Phycological Society of America, August 2007.

Graff, J. & **J. Rines** & D. Smith. Bacterial attachment to phytoplankton in Monterey Bay. Northeast Algal Society, April 2007.

HONORS/AWARDS/PRIZES

As documented in previous annual reports, Rines' technician and PhD student Malcolm McFarland has developed a semi-automated, epifluorescence and image-analysis based protocol for quantifying the picoplankton found in our samples, in order to ascertain the importance of these tiny cells to both the phytoplankton community, and the optical signals measured by Donaghay & Sullivan. He won the 'Best Student Poster' award at the 2007 Northeast Algal Society meeting for presentation of his methodology. This award carried a prize of $500 toward the cost of attending the 2007 Phycological Society of America meeting.